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Controlled thiol-initiated surface polymerization strategy for the preparation of hydrophilic polymer stationary phases

Dongping Yu[a], Aijin Shen[a], Zhimou Guo[*ab], Yukun Yan[c], Jingyu Yan[ab], Gaowa Jin [a] and Xinmiao Liang[*a]

[a] *Key Lab of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian,116023, P.R. China. E-mail: liangxm@dicp.ac.cn*

[b] *National Glycoengineering Research Cneter, Shandong University, Jinan,250012, P.R. China.*

[c] *Materials Fabrication and Processing Division, Institute of Metal Research, Chinese Academy of Sciences, Shenyang, 110016, P.R. China. E-mail: ykyan@imr.ac.cn*

Experimental details

Materials and regents

Spherical silica (5 μm particle size, 200 Å pore size, 150 m^2 g^{-1} surface area) was purchased from Fuji Silysia Chemical (Kasugai,Japan). Toluene (99.0%) was obtained from Kermel (Tianjin, China). 3-Mercaptopropyl trimethoxysilane (98%) was purchased from TCI (Tokyo, Japan). 1-Vinylimidazole (98%) and 2-Hydroxyethyl acrylate (99.5%) were purchased from Energy (Shanghai, China). Acrylic acid (99.5%) was provided by Alfa Aesar (Ward Hill, MA, USA). Acrylic amide (99.0%) was purchased from Aladdin (Shanghai, China). 4-Cyano-4-(phenylcarbono-thioylthio) pentanoic acid (CPA-DB) and 3-(methacryloylamino) propyl-dimethyl-(3-sulfopropyl) ammonium hydroxide (99%)was obtained from Innotech (Dalian, China). Five polar compounds uracil, uridine, cytosine, cytidine and orotic acid were purchased from Acros (Fair Lawn, NJ, USA). Six peptides Methionine enkephaline (96.41%), Angiotensin IV (97.91%), Bivalirudin (98.23%), Angiotensin II (97.49%), Angiotensin I (95.97%), Exenatide (99.36%) were purchased from GL Biochem (Shanghai, China). Monoclonal antibody (mAb), human serum albumin (HSA), bull serum albumin (BSA) cytochrome C (Cyt.C), sodium dihydrogen phosphate (NaH_2PO_4, 99.0%) and disodium hydrogen phosphate (Na_2HPO_4, 99.0%) were purchased from Sigma Aldrich (St.Louis, MO, USA). Melezitose, kestose and gentiano and nystose were kindly donated by Jiangnan University (Wuxi, PR China). Acetonitrile (ACN) and methanol (CH_3OH) of HPLC grade were obtained from Merck (Darmstadt, Germany). Water was purified using a Milli-Q purification system (Billerica, MA, USA). 2, 2'-azobis [2-methylpro-pionamidine] dihydrochloride (AIBA, 98%) and ammonium formate (97%) and formic acid (98%) were obtained from J&K Scientific (Beijing, China).

Chromatographic conditions

With 40 mL of methanol as slurry solvent and 80 mL of methanol as propulsion solvent under a pressure of 60 MPa, 2.0 of resulting material was slurry-packed into a stainless steel column (150 mm × 4.6 mm I.D.). The ZIC-HILIC column served as reference was obtained from Merk (Darmstadt, Germany). The C4 column (150 mm×4.6 mm i.d. particle size 5 μm, pore size 30 nm) was purchased from Acchrom (Beijin, China). The chromatographic system consisted of a 2695 HPLC pump, a 2489 ultraviolet-visible detector and a 2424 evaporative light scattering detection (ELSD) system. Data were collected and analyzed by Empower software version 3.0. These instruments and workstations were purchased from Waters (Milford, USA). For chromatographic evaluations, the flow rate was 1.0 mL min^{-1} and the column temperature was 30 °C unless otherwise specified. The mobile phase was composed of ammonium formate or NaH_2PO_4 aqueous solution (mobile phase A), acetonitrile (B), and water (C). The ELSD parameters were as follows: gain, 10; gas (N_2) pressure, 30 psi; nebulizer in heated mode at 75% (45 °C);

drift tube temperature, 80 ◦C.

Characterizations

The elemental analysis was performed on a Vario EL III elemental analysis system (Elementar, Hanau, Germany). ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (CP/MAS NMR) was performed on a Bruker AVANCE 500 MHz NMR spectrometer (11.7 T). Molecular weights and molecular weight distributions of polymers cleaved from silica were determined using a SHIMAZU gel permeation chromatograph (GPC) equipped with a LC20A HPLC pump, RID-10A refractive index detector, and Viscotek columns (A3000 Aq GPC/SEC in the effective molecular weight range of 1000-100000, respectively). The gel permeation chromatography (GPC) system was calibrated with dextranum standards obtained from National Institutes for Food and Grug Control with molecular weights ranging from 2500 to 86400.

Synthesis of hydrophilic polymer stationary phases

The mercaptopropyl modified silica (SH-Silica) was prepared according to the following procedures. 50 g of silica gel was dispersed in 150 mL of toluene, 30 mL of 3-mercaptopropyltrimethoxysilane and 10 mL of pyridine were added. Then mixture was refluxed for 24 h. The suspension was filtered and the solid was washed with toluene, methanol, water and methanol successively.

5 g of SH-Silica was added into the solution of AIBA, CPA-DB, and monomer in water-methanol (1:1. v/v), and vacuuming-bubbling nitrogen for three times to remove oxygen. The reaction was continued for 72 h at 55 ◦C. The resulting material was filtered, washed successively with water and methanol, then dried at 80 ◦C overnight. The resulting polymer stationary phase was obtained.

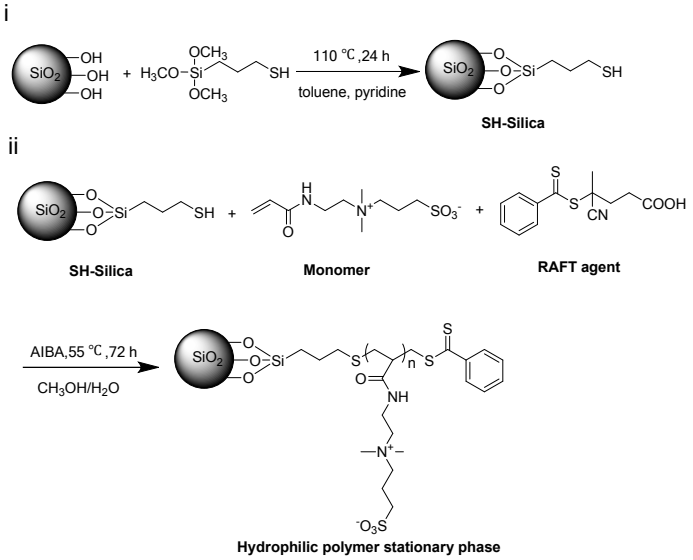

Fig. S1. The preparation of hydrophilic stationary phase

The TENS material was characterized by solid state ^{13}C/CPMAS NMR (Fig. S2). The signal at 214.93 ppm was assigned to the carbon atoms of thiocarbonyl groups. The signal at 176.34 ppm was assigned to the carbon atoms of carbonyl groups. The signals between 142 ppm and 123 ppm belonged to the carbon atoms of phenyl groups. The signals between 66 ppm and 12 ppm belonged to the rest carbon atoms of TENS polymer chains.

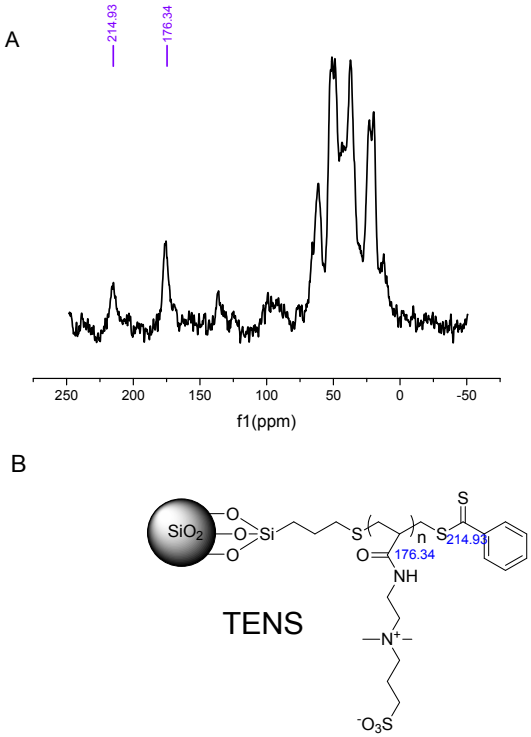

Fig. S2 Solid state ^{13}C CP/MAS NMR spectra of TENS material

Optimization of the polymerization condition

The reaction conditions of controlled thiol-initiated surface polymerization were investigated (shown in Table S1). As shown in Table S1, the amount of CPA-DB (RAFT agent) has a great influence on the carbon content, indicating that RAFT agents play an important role in controlling the thiol-initiated surface polymerization. The highest carbon content was obtained by **Entry 8**. Hence the hydrophilic polymer stationary phases were prepared under the condition of **Entry 8**.

Table SI. The optimization of controlled thiol-initiated surface polymerization

Entry	SH-Silica	Acrylamide	CPA-DB	AIBN	Reaction time	C %	N %
	g	g	g	g	h		
1	5	2.132	0.084	0.163	24	4.13	1.46
2	5	2.132	0.084	0.033	24	3.15	0.97
3	5	2.132	0.084	0.325	24	4.41	1.56
4	5	2.132	0.419	0.163	24	2.10	0.40
5	5	2.132	0.168	0.163	24	3.48	1.17
6	5	2.132	0.084	0.163	24	3.93	1.49
7	5	2.132	0.084	0.325	72	4.81	1.67
8	5	4.265	0.084	0.325	72	5.89	2.06
9	5	2.132	0.042	0.325	72	4.74	1.6

Determined by elemental analysis.

GPC analysis of the molecular weight distribution of polymer

1 g of resulting polymer grafted silica was dissolved in HF (2 mL, 40% in aq), after dissolved completely, adjusted pH to 7.0 by adding NaOH solutions (20% wt). The supernatant was desalted by mixed ion exchange column, concentrated and subjected to GPC analysis.

The grafted polymer on TEAM material was cleaved and analyzed by GPC. Meanwhile, a polyacrylamide stationary phase material prepared without RAFT agent (TEAM_0) was also investigated. The molecular weight distribution of polymers on TEAM was much narrower than TEAM_0 (Fig.S3).

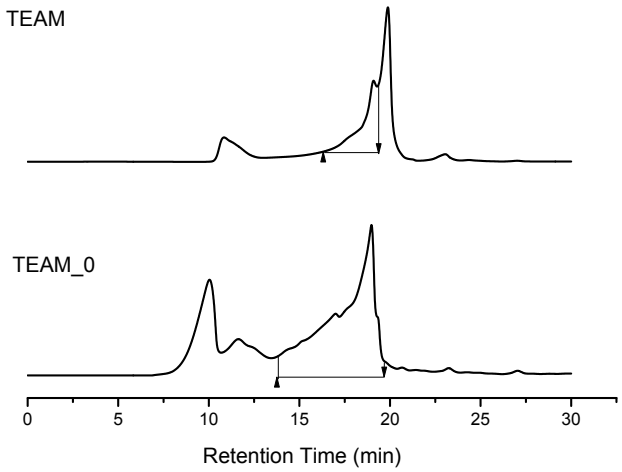

Fig. S3 GPC analysis of cleaved polymer on TEAM and TEAM_0 materials. TEAM: Mn=800, PDI=1.19; TEAM_0: Mn=1400, PDI=3.55

The adsorption properties of proteins on hydrophilic polymer materials

10 mg of resulting silica material was added into 0.5 mg/mL of protein solutions (0.5 mL, dissolved in 50 mM PBS, pH =7.0) and vibrated for 4 h (25 ℃). The supernatant was obtained after centrifugation and injected into the chromatographic system. At the same time, the protein solutions before adsorption was also injected into the chromatographic system to serve as a reference. The chromatographic conditions were as follows: C4 column (150 mm×4.6 mm i.d.); mobile phase A, ACN/formic acid (1000/1), mobile phase B, H_2O/formic acid(1000/1), gradient: 20%-80% A, 6 min; injection volume 10 μL; flow rate 1.5 mL min^{-1}; column temperature 30 ℃ ; detection wavelength 280 nm. The obtained peak area was used to calculate the adsorption amount of proteins (Δm, ng cm^{-2}) according to equation:

$$\Delta m = (A_0 - A_1)\, C_0 V_0 \times 10^5 / (A_1 m_1 S)$$

Where A_0 and A_1 is the peak area of protein solutions before and after adsorption respectively; C_0 is the concentration of protein solutions before adsorption, which is 0.5 mg mL^{-1}; V_0 is the volume of protein solutions, which is 0.5 mL; m_1 is the amount of resulting silica material, which is 10 mg; S is the surface area of the silica material, which is 150 m^2 g^{-1}.

Table S2. The adsorption amount of proteins on different silica materials

	Proteins	Before adsorption	After adsorption			
			SH-Silica	TEAM	TENS	TEHEA
Δm/ ng cm^{-2}	**mAb**	---	16.67	0	0	0
	HSA	---	12.68	0	0.06	0
	Cyt.C	---	16.60	0.58	0.40	0

Based on the protein non-fouling properties, the silica modified by hydrophilic polymer was applied as size exclusion chromatography (SEC) separation materials. As shown in Fig.S4, bull serum albumin (BSA) and its dimer were partly separated on TEHEA column under SEC mode.

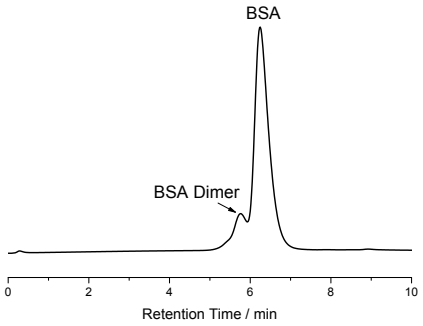

Figure. S4 The separation of BSA and its dimers under SEC mode on the TEHEA column (particle size 5 μm, pore size 30 nm, 150 mm×4.6 mm i.d.). Conditions: flow rate: 0.2 mL min^{-1}; 100 mM PBS solutions, pH=7.0; 30 ◦C; UV detection: 280 nm.

Comparison of chromatographic performance

In addition, a comparison of chromatographic performance between TENS column and ZIC-HILIC column was performed. In general, the ZIC-HILIC column has a similar chromatographic performance with TENS column. As shown in Fig.S5, saccharide isomers could achieve partly separation on ZIC-HILIC column, while they were achieved better separation on TENS column. However, in the separation of peptides under HILIC mode, peptides have stronger retention on ZIC-HILIC columns (Fig.S6). The hydrophilicity of ZIC-HILIC was weaker than TENS (shown in Fig.1), and it was speculated that there exists other interactions (such as electrostatic attraction) on ZIC-HILIC column, leading to the stronger retention of peptides.

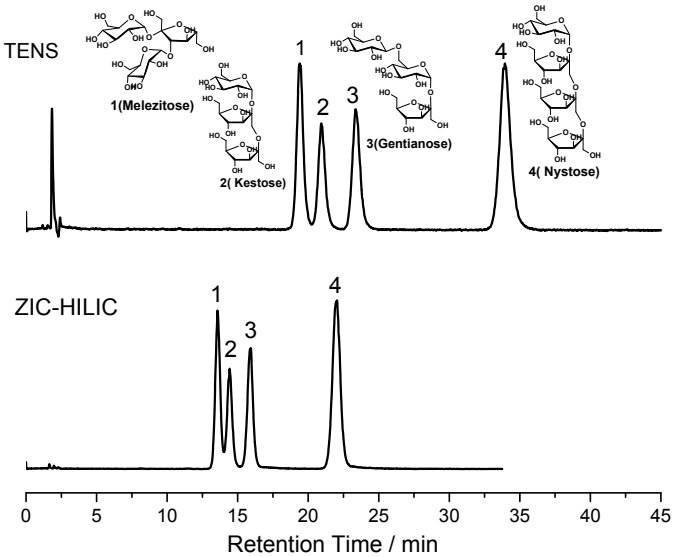

Fig. S5 The separation of saccharide isomers on the TENS column and ZIC-HILIC column (150 mm×4.6 mm i.d.). Conditions: flow rate: 1.0 mL min^{-1}; 30 °C; mobile phase, ACN/H_2O (80:20); evaporative light scattering detector (ELSD): gas pressure 30 psi, tube temperature 80 °C, gain 10.

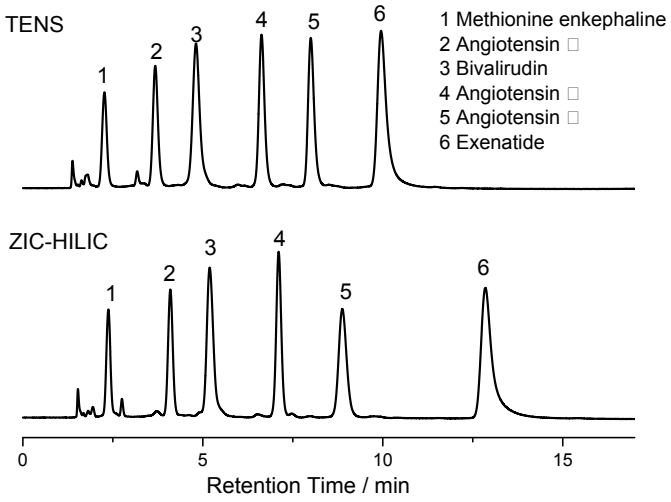

Fig. S6 The separation of six peptides on the TENS column and ZIC-HILIC column (150 mm×4.6 mm i.d.). Conditions: flow rate: 1.0 mL min^{-1}; 30 ^{o}C; mobile phase A, 50 mM NaH_2PO_4, pH 3.0; mobile phase B, ACN; mobile phase C, H_2O; gradient: 0-15 min, 10% A, 75% B, 15% C→10%A, 50% B, 40% C; UV detection: 210 nm.